

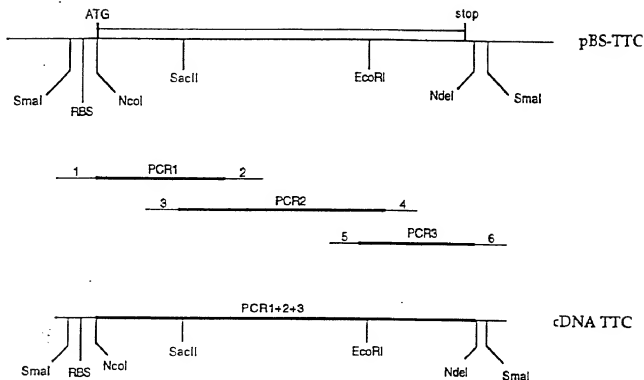
SK-ITC -> Genes

DNA sequence 1600 b.p. ggaaacagctat ... gtgcgtttacaa linear



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15 V D N E E D I D V I L K K S T I L N L D 38  
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23 I T N N D I S D I S G F N S S V I T Y P 58  
262 GAT GCT CAA TTG GTG CCC GGA ATA AAT GGC AAA GCA ATA CAT TTA GTA AAC AAT GAA TCT 321  
59 D A Q L V F G I N G K A I H L V N N E S 78  
322 TCT GAA GTT ATA GTG CAT AAA GCT ATG GAT AAT GAA TAT AAT GAT TTT AAT ATT TTT 381  
79 S E V I V H K A M D I E Y N D M F N N F 98  
382 ACC GTT AGC TTT TGG TTG AGG GTT CCT AAA GTA TCT GCT AGT CAT TTA GAA CAA TAT GGC 441  
59 T V S F W L R V P K V S A S H L E G Y G 118  
442 ACA AAT GAG TAT TCA ATA ATT AGC TCT ATG AAA AAA CAT AGT CTA TCA ATA GGA TCT GGT 501  
119 T N E Y S I I S S H K K H S L S I G Y G 138  
502 TGG AGT GTA TCA CTT AAA GGT AAT AAC TTA TGA TGS ACT TTA AAA GAT TCC GCG GGA GAA 561  
139 W S V S L K G N N L I W T L K D S A G E 158  
562 GTT AGA CAA ATA ACT TTT AGG GAT TTA CTT GAT AAA TTT AAT GCT TAT TTA GCA AAT AAA 621  
159 V R Q I T F R D L P D K F N A Y L A N K 178  
622 TGG GTT TTT ATA ACT ATT ACT AAT GAT AGA TTA TCT TCT GCT AAT TTG TAT ATA AAT GGA 681  
179 W V F I T I T N D R L S S A N L Y I N G 198  
682 GTA CTT ATG GGA AGT GCA AAA ATT ACT GGT TTA GGA CTT ATT AGA GAT GAT AAT ATA 741  
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219 T L K L L D R C N N N N Q Y V S I D K F R 238  
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239 I F C K A L N P K E I E K L Y T S Y L S 258  
862 ATA ACC TTT TTA AGA GAC TTC TGG GGA AAC CTT TTA GGA TAT GAT ACA TAT TTA 921  
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279 I P V A S S S K D V Q L K N I T D Y M Y 298  
982 TTG ACA AAT GCG CCA TCG TAT ACT AAC GGA AAA TTG AAT ATA TAT TAT AGA AGG TTA TAT 1041  
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1042 AAT GGA CTA AAA TTT ATT ATA AAA AGA TAT ACA CCT AAT AAT GAA ATA GAT TCT TTT GTT 1101  
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1102 AAA TCA GGT GAT TTT ATT AAA TTA TAT GTA TCA TAT AAC AAT AAT GAG CAC MIT GGA GGT 1161  
339 K S G D F I K L Y V S Y N N N E H I V G 358  
1162 TAT CCG AAA GAT GGA AAT GCC TTT AAT AAT CTT GAT AGA ATT CTA AGA GTA GGT TAT AAT 1221  
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379 A P G I F L Y K K N E A V K L R D L K T 398  
1282 TAT TCT GTA CAA CTT AAA TTA TAT GAT GAT AAA AAT GCA TCT TTA GGA CTA GTA GGT ACC 1341  
399 Y S V Q L L K L Y D D K N A S L G L V G T 418  
1342 CAT AAT GGT CAA ATA GGC AAC GAT CCA AAT AGG CAT ATA TTA ATT GCA AGC AAC TGG TAC 1401  
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439 F N H L K D K I L G C D W Y F V P T D E 458  
1462 GGA TGG ACA AAT GAT TAA aagattgatatctatgacatagtcgccgggatacctctagctgcagctcagggg 1535  
459 G W T N D \* (SEQ IDNO: 2) 464  
1536 ggggcccggtaacccaattccctatagtgatgcgtattcaattcctgcgcgtcttttcaaa (SEQ ID NO: 1) 1600

09816467-062901



#### TTC cDNA isolation:

The TTC cDNA was isolated from a *Clostridium Tetani* strain using Polymerase Chain Reaction. We used a three times PCR to generate three overlapping fragments respectively of 465 bp (PCR1; primer 1: 5'-CCC CCC GGG CCA CCA TGG TTT TTT CAA CAC CAA TTC CAT TTT CIT ATT C-3' & primer 2: 5'-CTA AAC CAG TAA TTT CTG-3'), of 648 bp (PCR2; primer 3: 5'-AAT TAT GGA CTT TAA AAG ATT CCG C-3' & primer 4: 5'-GGC ATT ATA ACC TAC TCT TAG AAT-3') and of 338 bp (PCR3; primer 5: 5'-AAT GCC TTT AAT AAT CTT GAT AGA AAT-3' & primer 6: 5'-CCC CCC GGG CAT ATG TCA TGA ACA TAT CAA TCT GTT TAA TC-3'), and each fragment was sequentially cloned into pBluescript KS+ to produce plasmid pBS-TTC. The upstream primer 1 contained the Ribosome Binding Site (RBS) and translation initiation signals.

FIGURE 2

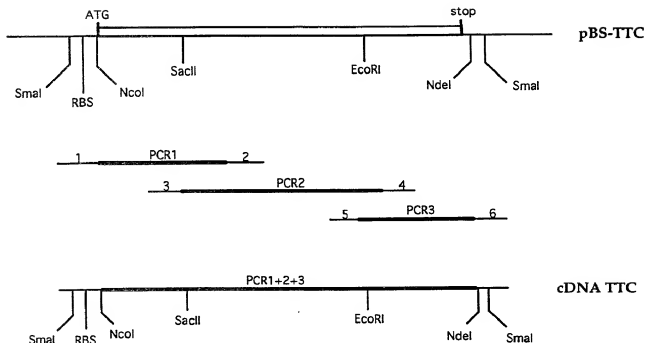
SK-TTC → Genes

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1	<u>M</u> V F S T P I P F S Y S K N L D C W	18	
142	GTT GAT AAT GAA GAA GAT ATA GAT GTT ATA TTA AAA AAG AGT ACA ATT TTA AAT TTA GAT	201	
19	V D N E E D I D V I L K K S T I L N L D	38	
202	ATT AAT AAT GAT AAT ATA TCA GAT ATA TCT GGG TTT AAT TCA TCT GTA ATA ACA TAT CCA	261	
39	I N N D I I S D I S G F N S S V I T Y P	58	
262	GAT GCT CAA TTG GTG CCC GGA ATA AAT GGC AAA GCA ATA CAT TTA AAC AAT GAA TCT	321	
59	D A Q L V P G I N G K A I H L V N N E S	78	
322	TCT GAA GTT ATA GTG CAT AAA GCT ATG GAT AAT GAA TAT AAT GAT ATG TTT AAT AAT TTT	381	
79	S E V I V H K A M D I E Y N D M F N F	98	
382	GTT AGC TTT TGG TTG AGG GTT CCT AAA GTA TCT GCT AGT CAT TTA GAA CAA TAT GGC	441	
99	T V S F W L R V P K V S A S H L E Q Y G	118	
442	ACA AAT GAG TAT TCA ATA ATT AGC TCT ATG AAA CAA CAT AGT CTA TCA ATA GGA TCT GGT	501	
119	T N E Y S I I S S M K H S L S I G S	138	
502	TGG AGT GTA TCA CTT AAA GGT AAT AAC TTA ATA TGG ACT TTA AAA GAT TCC GCG GAA	561	
139	W S V S L K G N N L I W T L K D S A G E	156	
562	GTT AGA CAA ATA ACT TTT AGG GAT TTA CCT GAT AAA TTT AAT GCT TAT TTA GCA AAT AAA	621	
159	V R Q I T F R D L P D K F N A Y L A N K	178	
622	TGG GTT TTT ATT ATT GAT AGA TTA TCT TCT GCT AAT TTT TAT ATA AAT GGA	681	
179	W V F I T I T N D R L S A N L Y I N G	198	
682	GTA CTT ATG GGA AGT GCA GAA ATT ACT GGT TTA GGA GCT ATT AGA GAG GAT AAT AAT ATA	741	
199	V L M G S A E I T G L G A I R E D N N I	218	

FIG. 1A

742 ACA TTA AAA CTA GAT AGA TGT AAT AAT AAT CAA TAC GTT TCT ATT GAT AAA TTT AGG 801  
219 T L K L D R C N N N N Q Y V S I D K F R 238  
802 ATA TTT TGC AAA GCA TTA AAT CCA AAA GAG ATT GAA AAA TTA TAC ACA AGT TAT TTA TCT 861  
239 I F C K A L N P K E I E K L Y T S Y L S 258  
862 ATA ACC TTT TTA AGA GAC TTC TGG GGA AAC CCT TTA CCA TAT GAT ACA GAA TAT TAT TTA 921  
259 I T F L R D F W G N P L R Y D T E Y Y L 278  
922 ATA GTA GCT TCT AGT TCT AAA GAT GTT CAA TTG AAA AAT ATA ACA GAT TAT ATG TAT 981  
279 I P V A S S S K D V Q L K N I T D Y M Y 298  
982 TTG ACA AAT GCG CCA TCG TAT ACT AAC GGA AAA TTG AAT ATA TAT TAT AGA AGG TTA TAT 1041  
299 L T N A P S Y T N G K L N I Y Y R L Y 318  
1042 AAT GGA CTA AAA TTT ATT ATA AAA AGA TAT ACA CCT AAT AAT GAA ATA GAT TCT TTT GTT 1101  
319 N G L K F I I K R Y T P N N E I D S F V 338  
1102 AAA TCA GGT GAT TTT ATT AAA TTA TAT GTA TCA TAT AAC AAT AAT GAG CAC ATT GTA GGT 1161  
339 K S G D F I K L Y V S Y N N E H I V G 358  
1162 TAT CCG AAA GAT GGA AAT GCC TTT AAT AAT CTT GAT AGA ATT CTA AGA GTA GGT TAT AAT 1221  
359 Y P K D G N A F N N L D R I L R V G Y N 378  
1222 GCC CCA GGT ATC CCT CTT TAT AAA AAA ATG GAA GCA GTA AAA TTG CGT GAT TTA AAA ACC 1281  
379 A P G I P L Y K K M E A V K L R D L K T 398  
1282 TAT TCT GTA CAA CTT AAA TTA TAT GAT AAT AAA AAT GCA TCT TTA GGA CTA GTA GGT ACC 1341  
399 Y S V Q L K L Y D D K N A S L G L V G T 418  
1342 CAT AAT GGT CAA ATA GGC AAC GAT CCA AAT AGG GAT ATA TTA ATT GCA AGC AAC TGG TAC 1401  
419 H N G Q I G N D P N R D I L I A S N W Y 438  
1402 TTT AAT CAT TTA AAA GAT AAA ATT TTA GGA TGT GAT TGG TAC TTT GTA CCT ACA GAT GAG 1461  
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1462 GGA TGG ACA AAT GAT TAA acagattgatgttcatgacatatccggggatcctctagatgcacctcgagg 1535  
459 G W T N D \* (SEQ ID NO:2) 464  
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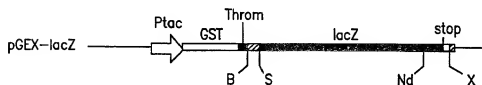
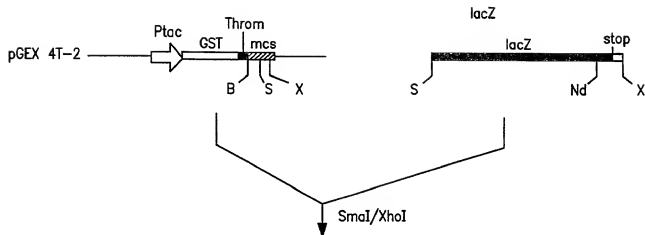
FIG. 1B



#### TTC cDNA isolation:

The TTC cDNA was isolated from a *Clostridium Tetani* strain using Polymerase Chain Reaction. We used a three times PCR to generate three overlapping fragments respectively of 465 bp (PCR1; primer 1: 5'-CCC CCC GGG CCA CCA TGG TTT TTT CAA CAC CAA TTC CAT TTT CTT ATT C-3' (SEQ ID NO:4) & primer 2: 5'-CTA AAC CAG TAA TTT CTG-3' (SEQ ID NO:5), of 648 bp (PCR2; primer 3: 5'-AAT TAT GGA CTT TAA AAG ATT CCG C-3' (SEQ ID NO:6) & primer 4: 5'-GGC ATT ATA ACC TAC TCT TAG AAT-3' (SEQ ID NO:7) and of 338 bp (PCR3; primer 5: 5'-AAT GCC TTT AAT AAT CTT GAT AGA AAT-3' (SEQ ID NO:8) & primer 6: 5'-CCC CCC GGG CAT ATG TCA TGA ACA TAT CAA TCT GTT TAA TC-3' (SEQ ID NO:9), and each fragment was sequentially cloned into pBluescript KS+ to produce plasmid pBS-TTC. The upstream primer 1 contained the Ribosome Binding Site (RBS) and translation initiation signals.

FIG. 2



PCR fragment NdeI/XhoI replacement: stop was replaced by NcoI site at the end of lacZ (amplification with primers GAL1/GAL2 between NdeI and XhoI sites)

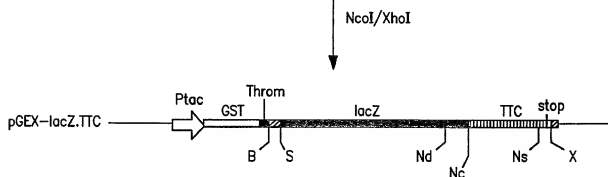
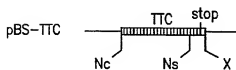
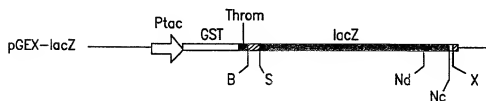
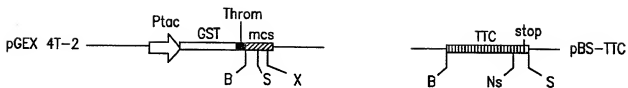
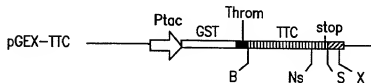


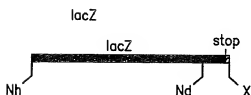
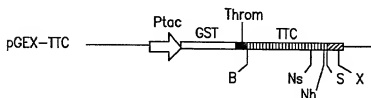
FIG. 3



BamHI/SmaI



PCR fragment NsiI/SmaI replacement: stop was replaced by NheI site at the end of TTC (amplification with primers TTC1/TTC2 between NsiI and SmaI sites)



NheI/XhoI

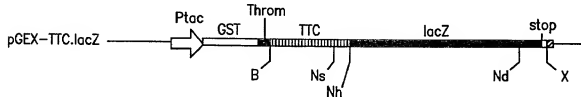


FIG. 4

pCMV.LACZ.TTC -> Graphic Map  
 DNA sequence 8519 b.p. tagttattaata ... accgccatgcac circular

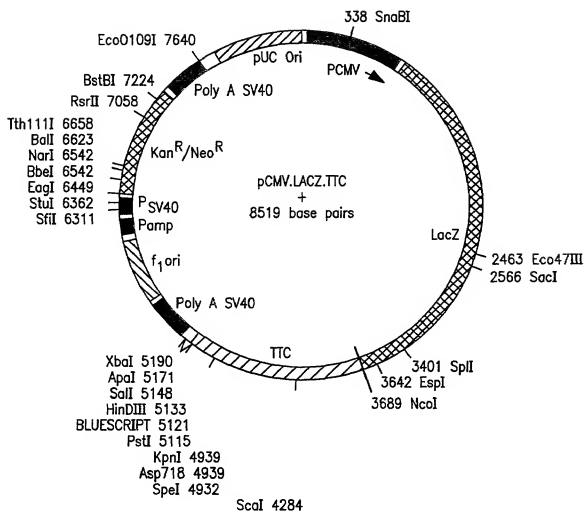


FIG. 5